2012-2013 Elk Brucellosis Surveillance and Research Summary

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Introduction

In the winter of 2010/11 Montana Fish, Wildlife and Parks (MFWP) initiated a multi-year surveillance and research project with the primary objectives of determining the geographical distribution of brucellosis in elk, evaluating movement patterns and the role they may play in the distribution and potential spread of brucellosis in Montana elk populations, and improving our understanding of the risk seropositive elk pose for transmission of *Brucella abortus* to cattle and other elk. The project was initiated due to concerns that brucellosis seroprevalence in elk appeared to be increasing in the Greater Yellowstone Area (GYA), the affected area may have expanded, and surveillance utilizing samples from hunter-harvested elk alone was not providing sufficient information to address questions about geographical distribution and seroprevalence in many areas (Anderson et. al 2010).

Prior to initiating this project, MFWP focused surveillance efforts in the GYA and utilized blood samples from hunter-harvested elk as the primary means of evaluating seroprevalence and brucellosis distribution. However, despite efforts to enhance surveillance, limited numbers of samples were obtained in key areas adjacent to the known distribution of brucellosis in elk. Questions were also raised about utilizing samples from hunter-harvested elk collected in the fall to assess transmission risk in mid- January through mid- June, the high risk period for *Brucella* transmission. Depending on the migratory behavior of an elk herd, a seropositive sample obtained in the fall during hunting season may not represent risk on winter and calving ranges when the transmission rate is the highest. Based on information obtained in this project and from prior research efforts (Hamlin and Ross 1990, Proffitt et al. 2012, Proffitt et al. 2013), elk in many of the herds in and around the GYA tend to move long distances

between summer and winter ranges. As a result, MFWP shifted to a targeted surveillance approach which involved capturing elk on winter range in high priority areas where additional information on presence of brucellosis was needed. Areas that were considered "high priority" were adjacent to the previously identified distribution of brucellosis, had large elk populations, had a significant livestock presence and prior surveillance was inadequate to address brucellosis presence or absence. Hunter-based surveillance has been scaled back, but may continue to be used in certain areas where capture is not planned, capture is not feasible, elk movement information suggests that hunter-harvested samples adequately identify risk during the winter and spring, or to help bolster sample sizes.

Recent management strategies proposed by the "Elk Management Guidelines in Areas with Brucellosis Working Group" and approved by the MFWP Commission, focus on maintaining separation of elk and cattle within the Designated Surveillance Area (DSA) (MFWP Brucellosis Working Group 2013). A primary goal of this project is to provide supportive information for management decisions associated with those guidelines. More information on the citizens working group is available on line at: http://fwp.mt.gov/fishAndWildlife/management/elk/areasWithBrucellosisWG/default.html. Information gained from this project also supports Montana Department of Livestock management strategies and regulations associated with the DSA for livestock. MFWP and Montana Department of Livestock work collaboratively to identify high priority areas for this project.

Study Area and Methods

Pioneer Mountains

In the winter of 2012/13 elk capture efforts focused on the southern (HD 329 and HD 331) and western (HD 332) Pioneer Mountains (Figure 1). This area is near the known distribution of brucellosis and the current DSA boundary. Although not adjacent to the known distribution of brucellosis, HD 332 in the western Pioneers was included in the surveillance due to possible seropositive results received in 4 out of 35 hunter-harvested samples tested in 2005. The varied results received on the panel of tests conducted (the four elk tested positive on 2-3 out of a panel of 6 tests) raised questions as to whether this was a true *Brucella* reaction or a non-specific reaction resulting in a false positive.

Adult female elk (yearling and older) were captured with a netgun fired from a helicopter. Captured elk were hobbled, blindfolded, placed in a holding bag to keep them sternal, and slung under the helicopter while being transported to a processing site. At the processing site, ground crews removed the elk from the bag and collected a blood sample. The blood sample was taken to a portable lab where it was spun in a centrifuge in order to separate and collect the serum. Serum was tested in the field utilizing the card test and, when feasible, the fluorescence polarization assay (FPA). Due to weather conditions, sensitivity of the FPA machine to temperature and movement, and the time it takes to obtain results, not all elk were tested using the FPA in the field. Additional serum samples were obtained from each elk and delivered to the Montana Department of Livestock, Diagnostic Laboratory (Diagnostic Laboratory) for testing. Final classification of sero-reactors was based on screening results obtained at the Diagnostic Laboratory. GPS collars programmed to collect a location every ½ hour and equipped with a drop off mechanism that allows the collar to release in January of the following year were place on 30

cow elk. Detailed movement information will be downloaded from the collars after they are retrieved January/February 2014. GPS collars also carry a VHF component so the collar can be relocated. Aerial locations were conducted approximately once a month to monitor general elk movements and determine fate of the elk.

Elk that tested positive for exposure to *Brucella* received a GPS collar and were tested to determine pregnancy status through the use of an ultrasound machine. If pregnant, a vaginal implant transmitter (VIT) was inserted to aid in locating abortion or birth sites. The VIT has a temperature sensitive switch that, when above 32° C or while implanted, emits a constant 40 pulses per minute (ppm). When the implant cools to below 28° C (when expelled) the pulse increases to 80 ppm and is interrupted by a Precise Event Transmitter (PET) code which provides an indication of how long the device has been expelled (below 28° C). Although the PET code can provide information on how long it has been since the VIT was expelled, if the implant is in the sun or daytime temperatures warm it to above 32° C the code will reset. When the VIT is emitting a pulse rate indicating it has been expelled or is no longer in close proximity to the cow elk, ground crews locate the implant, search for an abortion or birth site and collect samples in efforts to determine if *Brucella abortus* was shed during the birth event.

Seropositive Recaptures

Seropositive elk captured in the Blacktail/Sweetwater Hills area (HDs 324 and 326) in February 2011 and seropositive elk captured in the Sage Creek area (HD 325) in January 2012 (Figure 1) were recaptured in January 2013. Elk were captured through the use of a netgun fired from a helicopter, removed from the net, hobbled and blindfolded. A small ground crew comprised of MFWP personnel was ferried to the elk to exchange the GPS collar, collect a blood sample, determine pregnancy status through the use of an ultrasound machine, and fit pregnant elk with a VIT to aid in locating abortion or birth sites. The drop off mechanism was removed from these GPS collars so the collar remains attached to the elk until physically removed. GPS collars were scheduled to be removed after 2 years. Elk originally captured in the Blacktail/Sweetwater Hills area in 2011 will retain their collar until the winter of 2013/14. Elk originally captured in the Sage Creek area in 2012 received new collars which are scheduled to be exchanged in 2014/15.

Seropositive pregnant elk were tracked using telemetry equipment in order to monitor VIT and pregnancy status. Seropositive, pregnant elk were located approximately once a week from the air and approximately twice a week from the ground from February 1 through May 14. Ground locations were increased during the typical calving period from May 15 until parturition, which generally ends in mid June. When the VIT was expelled, field staff located the VIT and searched the area for the birth or abortion site. Samples associated with birth or abortion sites were collected to evaluate if *Brucella abortus* was being shed on the landscape. The VIT was swabbed and both the swab and VIT placed in culture media. Tissue and environmental samples associated with abortion or birth sites were swabbed and collected (swabs were placed in WHO media). All material associated with a birth or abortion site, including the VIT, was kept cool and delivered to the Diagnostic Laboratory for culture. Any isolates identified as being possible *Brucella* sp. were submitted to the National Veterinary Services Laboratory (NVSL) in Ames, IA for identification. Seropositive elk will be recaptured, retested and, if pregnant,

implanted with a VIT for a total of 5 years. After the fifth year of study the elk will be removed from the population and tissue samples cultured for *Brucella* to provide data on the relationship between seroprevalence and actual infection.

In 2013, an addition was made to the study design. Calves from seropositive cow elk were captured, if possible, and fitted with a VHF ear tag transmitter. These calves will be recaptured the following winter and bled to determine sero-status. The ear tag transmitter will be replaced with a VHF collar. These elk will be followed for 5 years to determine sero-status and sero-conversion rates. If seropositive, these elk will be collected at the completion of the study to investigate the relationship between serostatus and infection.

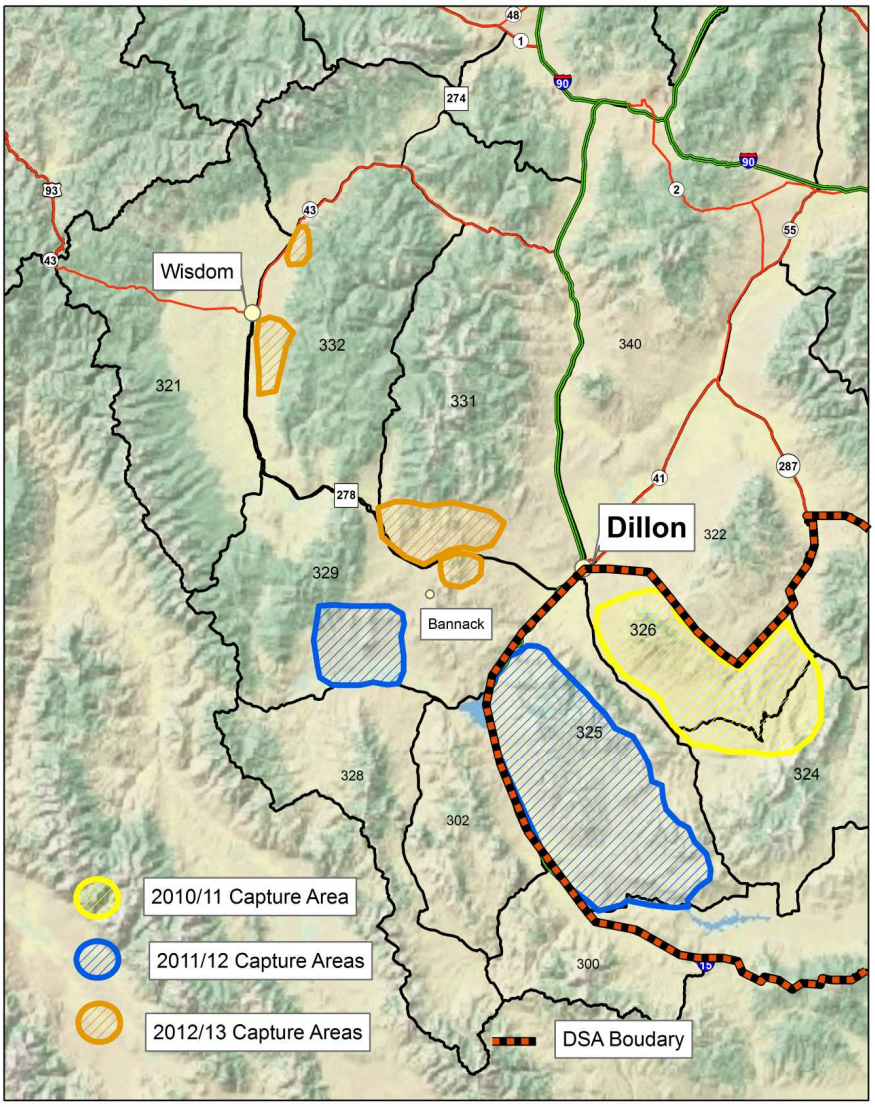

Figure 1. General elk capture locations for the elk brucellosis and research project, 2010-2013.

State-wide Surveillance

State-wide surveillance for brucellosis was conducted in conjunction with elk research projects in 2 locations of Montana. Blood was collected from adult female elk captured in the Bitterroot Mountains (HD 250 and HD 270) and in the Missouri Breaks (HD 621 and HD 622). Serum was extracted from the blood sample, submitted to the Diagnostic Laboratory, and screened for exposure to *Brucella*.

Blood collection kits were dispersed to hunters participating in the spring of 2013 brucellosis management hunts in HDs 317 and 560. Hunters were asked to collect blood samples from elk immediately after harvest and mail the samples to the MFWP Wildlife Laboratory. Serum was collected from samples suitable for testing and submitted to the Diagnostic Laboratory where it was screened for exposure to *Brucella*.

Results

Pioneer Mountains

One hundred adult female elk were captured and tested for exposure to Brucella in the field and at the Diagnostic Laboratory. Elk captures were distributed across 3 hunting districts in the foothills of the Pioneer Mountains with 19, 24, and 57 elk captured in HD 329, HD 331 and HD 332, respectively. No elk tested positive in the field or were classified as being positive based on screening tests conducted at the Diagnostic Laboratory. Since no elk tested positive for exposure to Brucella, no elk in the Pioneer Mountains study area received a VIT. Three elk died during capture operations. These elk were delivered to a local meat processor and the meat donated to the food bank.

Seropositive Elk

Eleven seropositive elk were recaptured for testing in late January, 2013. The majority of the elk were recaptured in the Blacktail/Sweetwater Hills or Sage Creek study areas. Six of the 7 seropositive elk originally captured in the Blacktail/Robb-Ledford area in the February 2011, were captured on the same winter range in 2013. One elk moved to the Wall Creek Wildlife Management Area in the winter of 2011/12. That elk again returned to Wall Creek and was recaptured there. Two of the 4 seropositive elk initially captured in the Sage Creek area in January, 2012 were recaptured in the Blacktail/Sweetwater Hills area in 2013. One of the elk died during capture operations. The elk was examined in the field and tissues were collected for culture. Tissues were delivered to the Diagnostic Laboratory and were culture negative for *Brucella* spp. The remaining 2 elk were recaptured in the Sage Creek area.

All recaptured seropositives remained seropositive for exposure to *Brucella* when retested in 2013. Eight of the 11 seropositive elk were pregnant, 1 of which was a mortality. The remaining 7 pregnant seropositives were fitted with a VIT and monitored for an abortion or birth event.

Ground and aerial tracking began on February 2, 2013. Elk were relocated from the ground on average between 1.8 and 3.5 times per week, and approximately once per week from the air until May 14. Ground tracking frequency increased slightly after May 14. Five elk expelled VITs ranging in dates from

approximately May 22 to June 8. The time period that elapsed from when the VIT was still implanted (slow mode) to the time when it was first heard on expelled (fast) mode ranged from 1 to 4 days. The birth occurred sometime during this time period with the PET codes ranging from 2.5 hrs to 43.5 hrs. With the exception of 1 birth site, all samples were collected on the same day the VIT was heard on fast mode. Collection of samples was delayed for 11 days for 1 birth site. Attempts to culture *B. abortus* from the VIT and environmental samples at the site where the VIT was expelled were unsuccessful. A summary of pregnancy status and birth site culture results is presented in Table 1. Live calves were observed near 2 of the sites. One calf was captured and fitted with an ear tag transmitter.

Table 1. Pregnancy status and birth site culture results for seropositive elk captured in the Blacktail/ Sweetwater Hills and Sage Creek study areas.

Elk ID #	Original Capture Location	2011 Pregnancy Status	2011 Birth Site Culture Results	2012 Pregnancy Status	2012 Birth Site Culture Results	2013 Pregnancy Status	2013 Birth Site Culture Result
BT10055	HD 324/326	Open	NA	Open	NA	Open	N/A
BT10045	HD 324/326	Open	NA	Pregnant	*B. abortus* (soil sample)	Pregnant	VIT failure
BT10068	HD 324/326	Pregnant	Negative	Pregnant	Negative	Pregnant	Negative
BT10075	HD 324/326	Open	NA	Open	NA	Open	NA
BT10058	HD 324/326	Pregnant	Negative	Pregnant	Negative	Pregnant	Negative
*BT10063	HD 324/326	Pregnant	Negative	Pregnant/ Mortality	*Negative	NA	NA
BT10083	HD 324/326	Pregnant	Negative	Pregnant	Negative	Pregnant	Negative
SC11097	HD 325	NA	NA	Pregnant	Negative	Mortality	*Negative
SC11050	HD 325	NA	NA	Pregnant	Negative	Pregnant	Did not expel VIT
SC11087	HD 325	NA	NA	Pregnant	*B. abortus* (fetal tissue)	Open	NA
SC11031	HD 325	NA	NA	Pregnant	Negative	Pregnant	Negative
SC11045	HD 325	NA	NA	Open	NA	Pregnant	Negative

*Represents culture results from tissues collected from a carcass.

Pregnancy status, detection of a birth site, and collection of samples was not completed for 2 of the seropositive, pregnant elk. The VIT malfunctioned and stopped emitting a pulse in 1 elk (BT10045) in mid- April. The cause of the malfunction is unknown. Ground crews searched the area the elk was frequenting in efforts to locate an aborted fetus in case an abortion event had occurred and the temperature switch in the implant malfunctioned after being expelled. No fetus was detected despite repeated efforts over several days to search the area. Ground crews increased efforts to observe this elk and determine if a calf was present by her side from the time the VIT malfunctioned through mid June. At no time was a calf observed.

The VIT was not expelled by elk SC11050. She was considered pregnant both on ultrasound evaluation in the field and by blood tests completed at a later date. The elk was last relocated on 10/1/2013 and she still retained the VIT. The reason for retaining the VIT is unknown.

Although many elk demonstrated site fidelity during the calving time period, the actual site where VITs were expelled varied across years (Figure 2). Many elk returned to the same general location they calved in during 2012, but several moved towards summer range before having their calf in 2013.

Statewide surveillance

Forty-one female elk were captured in the Bitterroot Mountains (HD 250 and HD 270) as part of an ongoing research project. Blood was collected from these elk and the serum tested for exposure to *Brucella* at the Diagnostic Laboratory. All 41 were classified as seronegative. From 2010 through 2013, 123 elk have been tested for exposure to brucellosis in the Bitterroot Mountains. No samples have been classified as seropositive to date.

Fifty-three elk were captured in the Missouri Breaks (HD 621 and HD 623) as part of a research project in 2013. Blood was collected and serum samples submitted to the Diagnostic Laboratory for Brucellosis screening. All samples were classified as seronegative for exposure to *Brucella*.

Blood samples were collected by elk hunters participating in the brucellosis elk distribution hunts in HD 317 south of Livingston and HD 560 south of Big Timber in March and April of 2013. Seven useable samples from HD 317 and 3 useable samples from HD 560 were screened for exposure to *Brucella* at the Diagnostic Laboratory. Three of 5 adult female elk were seropositive for exposure to *Brucella* in HD 317. The remaining samples were from calves and both were seronegative. All 3 samples from HD 560 (2 female and 1 male) were seronegative for exposure to *Brucella*.

Discussion

Pioneer Mountains

In 2005, 4 out of 35 elk blood samples received from hunters were positive on 2 out of a panel of 6 serologic tests used to detect brucellosis in HD 332. The individual tests that were positive are considered highly sensitive (highly sensitive tests detect a high proportion of animals that were truly exposed to *B. abortus*), but more specific tests (highly specific tests produce negative test results in a high proportion of animals that have not been exposed to *B. abortus*) were negative. At the time of

testing, the western blot test was used to evaluate if a cross-reaction due to exposure to other bacteria could have resulted in false-positive results. The western blot indicated that a cross-reaction may have occurred. However, subsequent information collected on culture positive elk suggested that the western blot was not able to accurately discern between true *Brucella* and cross-reactions in elk (Anderson et. al. 2010), but a non-specific or cross-reaction could not be ruled out. HD 332 resides on the west side of the Pioneers and is not adjacent to the known distribution of brucellosis in elk.

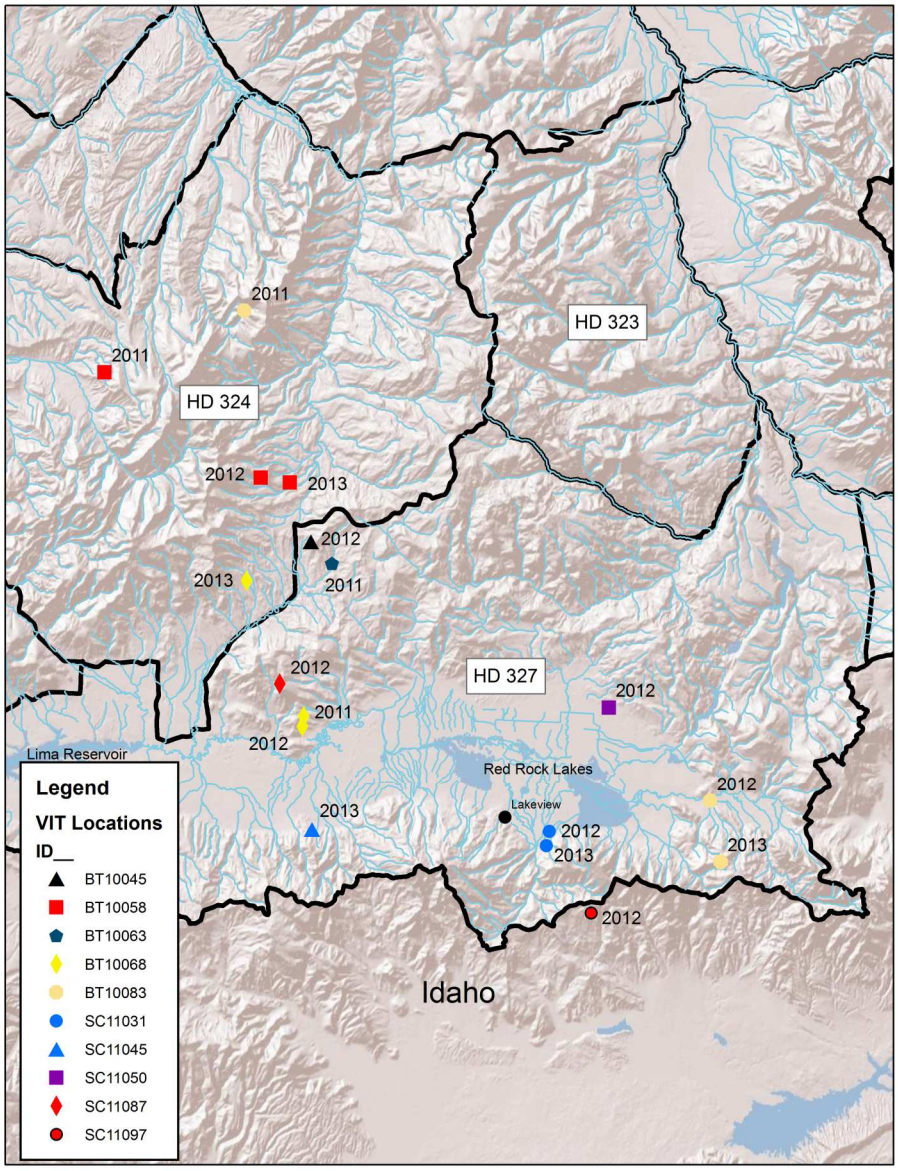

Figure 2. Locations where Vaginal Implant Transmitters (VITs) were recovered after birth events from seropositive elk, 2011-2013.

HDs 329 and 331 are in the southern Pioneer Mountains and adjacent to hunting districts to the west of where seropositive elk have been previously detected (HD 324 and HD 326) (Anderson et al. 2011). Sample efforts in HDs 329, 331 and 332 were conducted to help assess the status of brucellosis in the southern and western Pioneer Mountains.

All elk captured in the southern and western Pioneer Mountains in 2013 and elk captured south of the Pioneer Mountains in 2012 were classified as negative for exposure to *Brucella* based on serologic testing. In total 130 elk have been tested in the area since 2012. Although data from the GPS collars placed on elk captured in 2013 will not be available until late January 2014, aerial locations of these collars from February through July revealed no movement of animals into areas where seropositive elk are known to be present. This information suggests that *Brucella* is not present in the Pioneers and that the "weak" positive test results in 2005 were likely non-specific reactions.

Seropositive Elk

Ground crews made attempts to locate elk calves either at the birth site or with the cow for all seropositive pregnant elk. Elk calves were documented at birth sites for 2 of the pregnant elk (BT10068 and BT10083). An ear tag transmitter was placed in 1 of the calves. The other calf could not be captured. If the ear-tagged calf survives until winter it will be recaptured, fitted with a VHF radio collar and tested for exposure to *Brucella*. Birth site samples were collected within 2 days of the VIT being expelled for both of the birth sites. Samples collected were culture negative for *B. abortus*.

Two elk gave birth on private property where access was limited (SC11031 and SC11045). *B. abortus* was not cultured from either birth site. Sample collection for SC11031's birth site was delayed by approximately 11 days until permission was granted by the landowner to retrieve the implant. When the VIT was recovered a birth site could not be identified and the only samples collected consisted of the VIT and a swab of the VIT. If *B. abortus* was expelled during the birth event, it is unlikely it would be cultured from the implant 11 days later. Samples from SC11045's birth site were collected within four days, with the PET code indicating that 23 hrs had elapsed since the VIT was expelled. A calf could not be documented for either of these elk, but both exhibited behaviors consistent with having a calf nearby. Both elk remained in the approximate area where the VIT was expelled for several days, and where reluctant to leave the area when ground crews collected the implant or when observed from the air during relocation flights.

The implant from the BT10058 had wet birth material present on the implant, suggesting the event had occurred recently. The PET code indicated the implant and associated tissues were collected 2.5 hours after being expelled. A fetus could not be found at the birth site and no calf was observed with the cow elk at any time. The tissue and VIT were culture negative for *B. abortus*. The fate of the calf is not known, but there is no indication that it survived.

The cause of the failure of BT10045's VIT is unknown. Neither the implant nor a fetus could be located in the area where it was last heard. At no time was an elk calf observed by her side during multiple observations. Based on the inability to observe a calf and the timing of the VIT failure it is possible that BT10045 experienced a reproductive failure (aborted fetus or stillborn calf). The inability to find a birth

site or document a live calf is unfortunate as she aborted her fetus in the previous year and *B. abortus* was cultured from the site. In cattle, *B. abortus* typically causes an abortion in the first pregnancy after infection. Subsequent pregnancy may be carried to full term but occasional abortions or still births are possible. The disease appears to act similarly in bison (Ryan et al. 2009). Although abortions after initial infection has been documented in captive elk (Kreeger et al. 2002, Roffe et al. 2004), there currently is little information on the effect *B. abortus* has on subsequent pregnancies in free-ranging elk.

The implant from SC11050 has not been expelled to date, despite the elk being pregnant on ultrasound examination and being pregnant of blood tests conducted after capture. The cause of her reproductive failure is unknown, but reabsorption of the fetus early in pregnancy could have occurred. Although it is possible that both techniques used for evaluating pregnancy status were inaccurate, it is extremely unlikely. If still in place, the VIT will be removed when recaptured in the winter of 2013/14.

Statewide Surveillance

Although there is concern that the geographical distribution of brucellosis in elk populations may have changed, in Montana brucellosis has not been detected outside the Greater Yellowstone Area (GYA) since testing began in the late 1980's. The boundaries of brucellosis may have changed and seroprevalence increased within the GYA (Anderson et al. 2010), but to date the disease has not been detected outside the GYA. All elk tested in the Bitterroot Mountains and the Missouri Breaks in 2013 were classified as seronegative. Additional testing is planned over the next two years in additional areas of the state to further identify the geographical distribution of brucellosis in Montana elk.

The brucellosis management hunts that occurred in HD 317 and HD 560 were designed to alter elk distributions through the use of limited hunting opportunity. As a result, the number of samples received was small. Brucellosis is not known to exist in elk east of the Absaroka Mountain divide and the 3 samples collected from HD 560 were negative for exposure to *Brucella*. The small sample size provides some information but does not provide any level of statistical confidence that brucellosis is not present in the elk population. Additional testing will be required to improve our ability to assess presence or absence of the disease. The portion of HD 560 where the management hunt occurred is currently within the established DSA.

Brucellosis has been detected previously in HD 317 (Anderson et al 2010) and this hunting district is currently within the DSA boundary. Although the sample size was very small, detecting *Brucella* exposure in 3 of 5 adult female elk is concerning. MFWP will be conducting hunter surveillance in this district during the 2013 general hunting season to improve estimates of seroprevalence. Additional hunter surveillance is also planned for southeastern Montana during the 2013 general hunting season in response to the detection of brucellosis positive elk in the Bighorn Mountains of Wyoming in 2012.

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